# LIST OF U.S. CUSTOMS LABORATORY METHODS

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# U.S. CUSTOMS LABORATORY METHODS

## USCL METHOD 18-01

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## AOAC 920.75
## Separation of Fat in Cacao Products

### SAFETY PRECAUTIONS

*This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.*

### 1 SCOPE AND FIELD OF APPLICATION

Chocolate and chocolate preparations are classified in Chapter 18 of the Harmonized Tariff Schedule of the United States (HTSUS). This is one of two USCL methods for the determination and extraction of fat from chocolate products.

### 2 REFERENCE

***AOAC 920.75***
Separation of Fat in Cacao Products

# U.S. CUSTOMS LABORATORY METHODS

## USCL METHOD 18-02

Index

# AOAC 963.15
# Fat in Cacao Products
# Soxhlet Extraction Method
# (Office International du Cacao et du Chocolate-AOAC Method)

### SAFETY PRECAUTIONS

*This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.*

### 1 SCOPE AND FIELD OF APPLICATION

Chocolate and chocolate preparations are classified in Chapter 18 of the Harmonized Tariff Schedule of the United States (HTSUS). This is one of two USCL methods for the determination and extraction of fat from chocolate products.

### 2 REFERENCE

***AOAC 963.15***
Fat in Cacao Products
Soxhlet Extraction Method
(Office International du Cacao et du Chocolat-AOAC Method)

# U.S. CUSTOMS LABORATORY METHODS

## USCL METHOD 18-03 Index

## AOAC 931.105
## Cacao Mass (Fat-Free) of Chocolate Liquor

## SAFETY PRECAUTIONS

*This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.*

### 1 SCOPE AND FIELD OF APPLICATION

Chocolate and chocolate preparations are classified in Chapter 18 of the Harmonized Tariff Schedule of the United States (HTSUS). This method is used for the determination of chocolate paste (liquor) in chocolate products.

### 2 REFERENCE

***AOAC 931.105***
Cacao Mass (Fat-Free) of Chocolate Liquor

# U.S. CUSTOMS LABORATORY METHODS

## USCL METHOD 18-04

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# AOAC 980.13
# Fructose, Glucose, Lactose, Maltose, and Sucrose in Milk Chocolate Liquid Chromatographic Method

### SAFETY PRECAUTIONS

*This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.*

### 1 SCOPE AND FIELD OF APPLICATION

Chocolate and chocolate preparations are classified in Chapter 18 of the Harmonized Tariff Schedule of the United States (HTSUS). This method is used for the determination of sugar in chocolate products by high pressure liquid chromatography (HPLC). The sugars that can be determined include fructose, glucose, lactose, maltose and sucrose.

### 2 REFERENCE

***AOAC 980.13***
Fructose, Glucose, Lactose, Maltose, and Sucrose in Milk Chocolate Liquid Chromatographic Method

# U.S. CUSTOMS LABORATORY METHODS

## USCL METHOD 18-05

Index

# AOAC 920.82
# Sucrose in Cacao Products

(USCL Surplus 6/96)

**SAFETY PRECAUTIONS**

*This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.*

## 1 SCOPE AND FIELD OF APPLICATION

Chocolate and chocolate preparations are classified in Chapter 18 of the Harmonized Tariff Schedule of the United States (HTSUS). This method is used for the determination of sugar in chocolate products by polarization. The preferred method for determination sucrose in chocolate products is by high pressure liquid chromatography (HPLC) (see **USCL 18-04**) because nonfat milk solids (lactose) can be determined in the same analysis and hence shorten the total analysis time. With the advent of HPLC, this method is not routinely used and was placed in USCL surplus status June, 1996.

## 2 REFERENCE

***AOAC 920.82***
Sucrose in Cacao Products

# U.S. CUSTOMS LABORATORY METHODS

## USCL METHOD 18-06

Index

## AOAC 933.04
## Lactose in Milk Chocolate

(USCL Surplus 6/96)

### SAFETY PRECAUTIONS

*This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.*

### 1 SCOPE AND FIELD OF APPLICATION

Chocolate and chocolate preparations are classified in Chapter 18 of the Harmonized Tariff Schedule of the United States (HTSUS). This method can be used for the determination of lactose in chocolate products but does not distinguish between reducing sugars. The preferred method for the determination of lactose in chocolate products is by high pressure liquid chromatography (HPLC) (see**USCL 18-04**). With the advent of HPLC, this method is not routinely and was placed in USCL surplus status June, 1996.

### 2 REFERENCE

***AOAC 933.04***
Lactose in Milk Chocolate

# U.S. CUSTOMS LABORATORY METHODS

## USCL METHOD 18-07

Index

## AOAC 945.34
## Fat (Milk) in Milk Chocolate

## SAFETY PRECAUTIONS

*This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.*

## 1 SCOPE AND FIELD OF APPLICATION

Chocolate and chocolate preparations are classified in Chapter 18 of the Harmonized Tariff Schedule of the United States (HTSUS). There are three methods that can be used for the determination of buttefat in chocolate products. One is a gas chromatographic (GC) method and it is the fastest. It should be used as a screening tool for all chocolate samples. However, since it is not a recognized method of a standards organization, the distillation must be performed when the results of the GC analysis will change the classification or quota as entered by the importer.

This method involves a distillation of the saponified extracted fat to determine a Reichert-Meissl value and the comparison of that value with the Reichert-Meissl value of an "authentic" butterfat. This is an empirical method and the total quantities of volatile fatty acids, soluble and insoluble in water, present in the fat are not directly determined. The accuracy of the results are dependent on strict adherence to the dimensions of the apparatus and the detail of the procedure.

## 2 REFERENCE

***AOAC 945.34***
Fat (Milk) in Milk Chocolate

# U.S. CUSTOMS LABORATORY METHODS

## USCL METHOD 18-08

Index

# AOAC 920.80
# Milk Fat in Milk Chocolate

### SAFETY PRECAUTIONS

*This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.*

### 1SCOPE AND FIELD OF APPLICATION

Chocolate and chocolate preparations are classified in Chapter 18 of the Harmonized Tariff Schedule of the United States (HTSUS). There are three methods that can be used for the determination of buttefat in chocolate products. One is a gas chromatographic (GC) method and it is the fastest. It should be used as a screening tool for all chocolate samples. However, since it is not a recognized method of a standards organization, the distillation must be performed when the results of the GC analysis will change the classification or quota as entered by the importer. This method involves a distillation of the saponified extracted fat to determine a Reichert-Meissl value and the comparison of that value with the Reichert-Meissl value of an “authentic” butterfat. This is an empirical method and the total quantities of volatile fatty acids, soluble and insoluble in water, present in the fat are not directly determined. The accuracy of the results are dependent on strict adherence to the dimensions of the apparatus and the detail of the procedure.

### 2 REFERENCE

***AOAC 920.80***
Milk Fat in Milk Chocolate

# U.S. CUSTOMS LABORATORY METHODS

## USCL METHOD 18-09 INDEX

# Butterfat in Chocolate Products

**SAFETY PRECAUTION**

*This method does not purport to address all the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use*

## 1 SCOPE AND FIELD OF APPLICATION

This is a modified AOAC method for the determination of butterfat. Reichert-Meissl-Polenske values are determined according to AOAC 15, 925.441 and Kirschner values (AOAC 8, 26.28). The amount of butterfat is then calculated using an empirical formula developed by the original authors of the Reichert-Meissl distillation. This calculation is based on a fixed value for butyric acid in butterfat.[1] Using this method has eliminated the problem of obtaining an "authentic" butterfat reference sample for comparison with the sample.

## 2 REFERENCES

**USCL 04-18**
***AOAC 925.41***
Acids (Volatile) in Oils and Fats
(Reichert-Meissl and Polenske Values)
Titrimetric Method

**USCL 08-01**
***AOAC 920.75***
Separation of Fat in Cacao Products

***AOAC 26.28*** (8th Edition, 1955)
Acids (Volatile) in Oils and Fats
Reichert-Meissl, Polenske and Kirschner Values

***Oil, Fats and Fatty Foods***
K.A. Williams
American Elsevier Publishing Co., Inc.
New York, 1966, p. 146

## 3 DEFINITIONS

**3.1** Reichert-Meissl value (RM) is the number of milliliters of 0.1 N aqueous alkali that are required to neutralize the water-soluble fatty acids distilled from 5 grams of the fat under the precise conditions of the method. (These acids are chiefly butyric and caproic acids).

**3.2** Polenske value is the number of milliliters of 0.1 N aqueous alkali that are required to neutralize the water-insoluble volatile acids distilled from 5 grams of fat under the precise conditions specified in the method. (These are mostly the higher fatty acids: caprylic, capric and lauric acids. Myristic and palmitic acids also contribute.

**3.3** Kirschner value is the number of milliliters of 0.1 N aqueous alkali that are required to neutralize the water-soluble volatile fatty acids which form water-soluble silver salts distilled from 5 grams of the fat under the precise conditions specified in the method. (This is

essentially a measure of butyric acid.)

## 4 SAMPLE PREPARATION

**4.1** Use the fat extracted using the "total fat" procedure in AOAC 920.75.

## 5 PROCEDURE

**5.1** The apparatus is depicted in AOAC 925.41.

**5.2** Follow the analysis procedure presented in AOAC 925.41. This procedure will yield the Reichert-Meissl and Polenske values only.

**5.3** The procedure for the Kirschner value is found in AOAC 26.28. Use 0.1 N NaOH instead of $Ba(OH)_2$ as specified in the AOAC procedure.

## 6 CALCULATIONS

**6.1** Reichert-Meissl Value

$$RM = [1.1 \times N \times (RM_{mL} - B_{mL}) \times 5] \div [0.1 N_{NaOH} \times W_S]$$

where

| | | |
|---|---|---|
| N | = | Normality of the NaOH solution |
| $RM_{mL}$ | = | Milliliters of base to neutralize water soluble acids |
| $B_{mL}$ | = | Milliliters of base to neutralize blank |
| $W_s$ | = | Weight of the fat in grams |

**6.2** Polenske Value

$$Pol = [(Pol_{mL} - B_{mL}) \times N \times 5] \div [0.1 N_{NaOH} \times W_S]$$

where

| | | |
|---|---|---|
| N | = | Normality of the NaOH solution |
| Pol | = | Milliliters of base to neutralize water insoluble acids |
| $B_{mL}$ | = | Milliliters of base to neutralize blank |
| $W_s$ | = | Weight of the fat in grams |

**6.3** Kirschner Value

$$K = [(K_{mL} - B_{mL}) \times (1000 + RM) \times 121] \div [10.000]$$

where

| | | |
|---|---|---|
| $K_{mL}$ | = | Milliliters of base to neutralize water insoluble acids which form soluble silver salts |
| $RM_{mL}$ | = | Milliliters of base to neutralize water soluble acids |
| $B_{mL}$ | = | Milliliters of base to neutralize blank |
| $W_s$ | = | Weight of the fat in grams |

**4.5** Percent Butterfat in the Fat

$$\%Butterfat = [K - 0.1 Pol - 0.24] \div [0.244]$$

# U.S. CUSTOMS LABORATORY METHODS

## USCL METHOD 18-10 INDEX

## Determination of Butyric Acid - Gas Chromatographic Method.

### SAFETY PRECAUTIONS

*This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.*

### 1 SCOPE AND FIELD OF APPLICATION

A gas chromatography (GLC) method for quantitation of water-soluble fatty acids from saponification and acidification of fat provides a convenient method for butterfat determination. It was determined that 3.59% butyric acid is an appropriate estimate for butyric acid content of pure butterfat (**7.1,7.2**).

### 2 REAGENTS AND MATERIALS

**2.1 Reagents**

**2.1.1** Alcoholic potassium hydroxide solution. 0.5 m/L in ethanol.

**2.1.2** o-Phosphoric acid solution. 5% (v/v).

**2.1.3** n-Butyric acid solution. 400 mg/L of redistilled butyric acid, solution freshly prepared.

**2.1.4** n-Valeric acid solution. 1000 mg/L of valeric acid (free of butyric acid), solution freshly prepared.

**2.2 Materials**

**2.2.1** Whatman No.1 filter paper, 9 cm diameter.

**2.2.2** Microbore syringe capable of delivering 0.5 microliter of liquid into injection port of gas chromatograph.

### 3 Apparatus

**3.1** Gas Chromatograph with optional capillary inlet system and flame ionization detector (FID).

**3.2** Column: Carbowax 20M, fused silica, 30 meter length, 530 micron diameter.

**3.3** Oven Temperature: 145° C. Isothermal.

**3.4** Carrier Gas: Helium. 30 mL/min. Splitless mode.

**3.5** Note: Any gas chromatographic column and conditions that produce baseline resolution of butyric and valeric acids are

acceptable.

## 4 SAMPLES

**4.1** Test Sample

**4.1.1** If necessary to extract fat from the commodity for which butterfat is to be determined, follow the prescribed method in the AOAC Official Methods of Analysis 15th Edition (7.3) for the particular commodity of interest (i.e. cheese, chocolate, etc).

**4.2** Test Portion

**4.2.1** Portion of fat from **4.1.1** to be analyzed.

## 5 PROCEDURE

**5.1** Test Portion

**5.1.1** Weigh 0.10-0.15 grams of the test sample (**4.1.1**) to the nearest 0.1 mg into a 50 mL beaker.

**5.2** Determination

**5.2.1** Add 3 mL of alcoholic potassium hydroxide solution (**2.1.1**) to the test portion (**5.1.1**).

**5.2.2** Cover the beaker with a watch glass, and heat on a steam bath for 10 min.

**5.2.3** Remove the watch glass and continue heating until the ethanol has completely evaporated.

**5.2.4** Allow the beaker to cool; accurately pipette 5.0 mL of water into the beaker.

**5.2.5** Cover with the watch glass. Swirl gently until the potassium salts of the fatty acids have dissolved.

**5.2.6** Accurately pipette 5.0 mL of the phosphoric acid solution (**2.1.2**) into the beaker.

**5.2.7** Swirl gently to coagulate the precipitated fatty acids.

**5.2.8** Filter through a 9 cm, No.1 Whatman filter paper (**2.2.1**) into a test tube with minimum capacity of 15 mL.

**5.2.9** Immediately prepare the filtrate for gas chromatography as indicated below.

**5.2.10** Accurately pipette 5.0 mL of the filtrate (**5.2.8)** into a container of minimum capacity of 10 mL.

**5.2.11** Accurately add 2.0 mL of the valeric acid solution (**2.1.4**).

**5.2.12** Mix and inject 0.5 mL into the gas chromatograph (**3**).

**5.2.12.1** The first peak, which goes off scale, is due to a residual trace of ethanol which is not completely evaporated on the steam bath.

**5.2.12.2** N-Butyric acid is eluted next.

**5.2.12.3** N-Valeric acid is the third peak.

**5.3** Calibration

**5.3.1** Prepare a standard solution by mixing 5.0 mL of the 400 mg/L butyric acid solution (**2.1.3**) with 2.0 mL of the 1000 mg/L valeric acid solution (**2.1.4**).

**5.3.2** Inject 0.5 microliter into the gas chromatograph.

## 6 EXPRESSION OF RESULTS

**6.1** Method of Calculation

**6.1.1** The response of a calibration curve for peak area ratios of butyric acid/valeric acid (used as an internal standard) vs concentration of butyric acid was found to be linear (7.1,7.2). Due to this linearity, the concentration of butyric acid in a sample may be calculated directly from the following typical internal standard formula.

**6.1.2**

$$R_f = (A_{rs}/A_{ist})x(W_{is}/W_{rs})$$

$$\% \text{ Butyric acid} = (A_s/A_{is})x(W_{is}/W_s)x1/R_f x100$$

Where:

$A_{is}$ = Internal Standard (Valeric Acid) area in the sample

$A_{isr}$ = Internal Standard (Valeric Acid) area in the standard

$A_{rs}$ = Reference Standard (Butyric Acid) area

$A_s$ = Butyric Acid area in the sample

$W_{is}$ = Weight of Internal Standard (Valeric Acid) which is equal to concentration of Internal Standard ($C_{is}$) multiplied by the volume of internal standard solution added ($V_{is}$)

$W_{rs}$ = Weight of Reference Standard Butyric Acid used to determine the response factor $R_f$. $W_{rs}$ is equal to concentration of Reference Standard ($C_{rs}$) multiplied by volume of Reference Standard used to determine $R_f$

$W_s$ = Weight of Sample (fat). It is equal to the concentration of sample ($C_s$) multiplied by the volume of the extract used for analysis. The final volume of the sample is 10 mL.

The percent butterfat in fat is given by:

$$\%\,Butterfat = \frac{\%\,Butyric\,Acid}{3.59\%} x100$$

The percent butter in the sample is given by:

% Butterfat in the sample =

(% Butterfat in fat
X
% Total Fat in Sample)/100

## 7 Bibliography


**7.1** Pearson, David, ***The Chemical Analysis of Foods***, First American Edition, 1971, Chemical Publishing Co., Inc., N.Y., N.Y., p.482-485.

**7.2** ***Gas Chromatography in Food Analysis,*** 1976, Butterworths, Boston, MA, p.148.

**7.3** ***AOAC Official Methods of Analysis,*** 15th Edition, Volume 2, 1990. See fat extraction references for individual commodities.

# U.S. CUSTOMS LABORATORY METHODS

## USCL METHOD 18-11

Index

# AOAC 935.33
# Silver Number for Detection of Coconut and Palm Kernel Oils



## SAFETY PRECAUTIONS

*This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.*

## 1 SCOPE AND FIELD OF APPLICATION

Chocolate and chocolate preparations are classified in Chapter 18 of the Harmonized Tariff Schedule of the United States (HTSUS). HTSUS 1806.20.6000 requires that confectioners' coatings contain more than 15% vegetable fat other than cocoa butter. Qualitatively, other vegetable fat can be distinguished from cocoa butter either by determining the "Silver Number" of the fat or by looking at the FAME (Fatty Acid Methyl Ester) profile of the fat. Typically cocoa butter has a silver number of less than 1 and coconut or palm kernel oil has a value greater than 20. Butterfat has a value of about 17 so it may be difficult to determine other vegetable fats in the presence of butterfat. In those cases, the GC FAME profile is the recommended method (**USCL 18-12**).

## 2 REFERENCE

# U.S. CUSTOMS LABORATORY METHODS

## USCL METHOD 18-12 INDEX

## GC FAME Profile: Other Vegetable Fat Determination

### SAFETY PRECAUTIONS

*This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.*

### 1 SCOPE AND FIELD OF APPLICATION

HTS 1806.20.6000 requires that confectioners' coating contain more than 15% vegetable fat other than cocoa butter. Qualitatively, other vegetable fat can be distinguished from cocoa butter either by determining the "Silver Number: of the fat or by looking at the FAME (Fatty Acid Methyl Ester) profile of the fat. Typically cocoa butter has a silver number of less than 1 and coconut or palm kernel oil has a value greater than 20. The problem is that butterfat has a value of about 17, so it may be difficult to determine other vegetable fats in the presence of butterfat. By acquiring a GC FAME profile, the presence of large amounts (greater than 15%) of other vegetable fat can be detected. The vegetable fats added to make confectioners' coatings are "lauric acid" fats like coconut or palm kernel oils to harden the product. The FAME profile of such a mixture will have a very significant lauric acid C 12 peak. Since cocoa butter contains no lauric acid and butterfat contains a much smaller amount of lauric acid, the presence of a large C12 component identifies the fat as containing a vegetable fat other than cocoa butter.

### 2 REFERENCES

**USCL 04-24**
***ASTM D 2800***
Test Method for Preparation of Methyl Esters from Oils for Determination of Fatty Acid Composition by Gas Chromatography

**USCL 04-25**
***ASTM D 1983***
Test Method for Fatty Acid Composition by Gas-Liquid Chromatography of Methyl Esters

**USCL 12-01**
***AOAC 963.22***
Methyl Esters of Fatty Acids in Oils and Fats
Gas Chromatographic Method
(AOAC-IUPAC Method)

# U.S. CUSTOMS LABORATORY METHODS

## USCL METHOD 18-13 Index

# AOAC 980.14
# Theobromine and Caffeine in Cacao Products
# Liquid Chromographic Method

### SAFETY PRECAUTIONS

*This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.*

### 1 SCOPE AND FIELD OF APPLICATION

Chocolate and chocolate preparations are classified in Chapter 18 of the Harmonized Tariff Schedule of the United States (HTSUS).

### 2 REFERENCE

***AOAC 980.14***
Theobromine and Caffeine in Cacao Products
Liquid Chromographic Method